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# Environmental Assessment of Buccaneer Gas and Oil Field in the Northwestern Gulf of Mexico 1978-1979. Volume IV. Bacterial Communities

(U.S.) National Marine Fisheries Service
Galveston, TX

Prepared for

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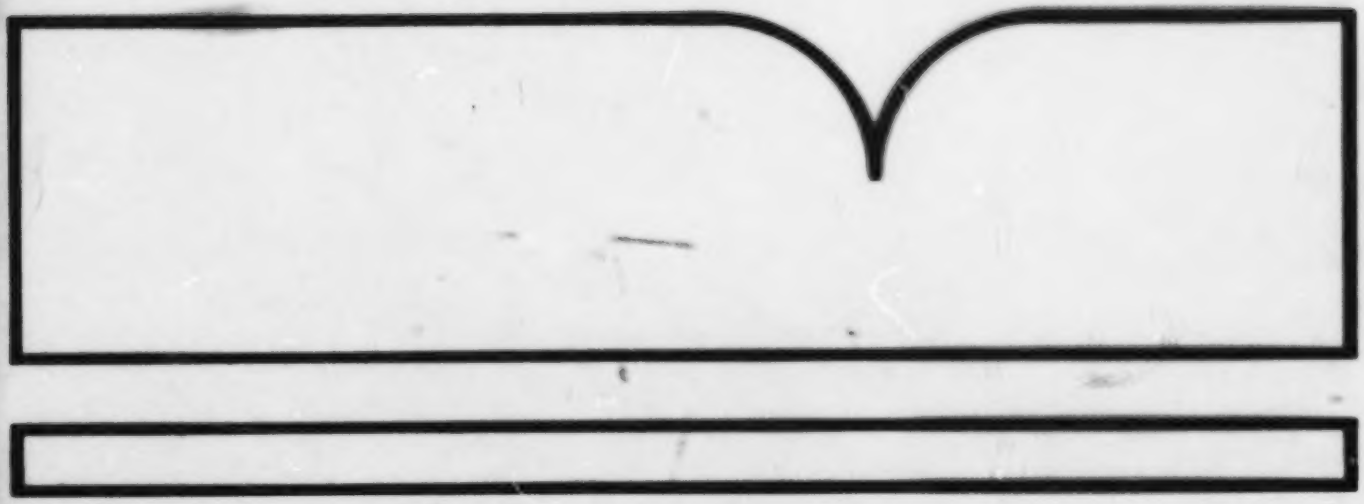

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16. ABSTRACT

Bacterial numbers and taxonomic types were found to be the same in the oil field as in the control area. No difference in bacterial diversity or biomass was n noted between stations but a change did occur between seasonal samples. Members of the genera Vibrio, Pseudomonas, Aeromonas and Acinetobacter were found to predominate in the sediment and fish samples. Vibrio sp. were found to be commonly associated with healthy and some diseased fish. Aeromonas hydrophilia was the only microorganisms consistently associated with the diseased fish collected in the oil field. The bacterial population of the oil field sites contained more oil degrading and sulfur oxidizing bacteria than the control site. Sulfate reducing bacteria were especially abundant in the sediment of the platform sites. The oil field isolates were able to grow readily in Buccaneer brine discharge and some isolates were stimulated by high concentrations of the brine discharge. Both mixed and some pure cultures of Buccaneer field bacteria were able to degrade the alkane portion of Buccaneer condensate. Combined with weathering, some bacterial culture was found to degrade the majority of the alkanes ~~under laboratory conditions in a ten day period. (Author modified)~~

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## Environmental Assessment of Buccaneer Gas and Oil Field In the Northwestern Gulf of Mexico, 1978-1979

## VOL. IV - BACTERIAL COMMUNITIES


BY

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A report to the Environmental Protection Agency on work conducted under provisions of Interagency Agreement EPA-IAG-D5-E693-EO during 1978-1979.



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Volume IV - BACTERIA

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## LIST OF VOLUMES

This Annual Report is printed in ten separate volumes:

Volume I - SYNOPSIS/DATA MANAGEMENT

Work Unit 2.6.1 — Synopsis

NMFS/SEFC Galveston Laboratory

Principal Investigators

Work Unit 2.2.3 — Implement, Monitor, and Modify Data Management System

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Volume II - SEDIMENTS AND PARTICULATES

Work Unit 2.3.2 — Investigations of Surficial Sediments and Suspended Particulates at Buccaneer Field

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Volume III - FISHES AND MACROCRUSTACEANS

Work Unit 2.3.5 — Effect of Gas and Oil Field Structures and Effluents on Pelagic and Reef Fishes, Demersal Fishes, and Macrocrustaceans

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Volume IV - BACTERIA

Work Unit 2.3.7 — Bacterial Communities

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Volume V - FOULING COMMUNITY

Work Unit 2.3.8 — Effects of Gas and Oil Field Structures and Effluents on Fouling Community Production and Function

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Volume VI - CURRENTS AND HYDROGRAPHY

Work Unit 2.3.9 — Currents and Hydrography of the Buccaneer Field and Adjacent Waters

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Volume VII - HYDROCARBONS

Work Unit 2.4.1 — Hydrocarbons, Biocides, and Sulfur

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Volume IX - FATE AND EFFECTS MODELING

Work Unit 2.5.1 — Sources, Fate and Effects Modeling

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Volume X - HYDRODYNAMIC MODELING

Work Unit 2.5.2 — Hydrodynamic Modeling

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## GUIDE TO USERS OF THE ANNUAL REPORT

Volume I (SYNOPSIS/DATA MANAGEMENT) of the Annual Report is designed to be used as a briefing document and as a key to more detailed scientific and technical information contained in Volumes II through X. Objectives, methods and results for each work unit are summarized in greatly abbreviated form within Volume I to facilitate dissemination of information. Thus, Volume I can be used alone or as a reference to companion Volumes II through X. Complete citations for literature cited in Volume I can be found in the Volumes II through X in which the detailed work unit reports are presented.

It is hoped that such an approach to environmental impact information dissemination will make the Annual Report a more useful and widely read document.

# FOREWORD

Increased petroleum development of the outer continental shelf (OCS) of the United States is anticipated as the U.S. attempts to reduce its dependency on foreign petroleum supplies. To obtain information concerning the environmental consequences of such development, the Federal Government has supported major research efforts on the OCS to document environmental conditions before, during, and after oil and gas exploration, production, and transmission. Among these efforts is the Environmental Assessment of Buccaneer Gas and Oil Field in the Northwestern Gulf of Mexico, a project funded by the Environmental Protection Agency (EPA) through interagency agreement with the National Oceanic and Atomospheric Administration (NOAA) and managed by the National Marine Fisheries Service (NMFS), Southeast Fisheries Center (SEFC), Galveston Laboratory, in Galveston, Texas. Initiated in the autumn of 1975, the study is now in its last year. Its major products have been annual reports disseminated by the National Technical Information Service, data files archived and disseminated by NOAA's Environmental Data and Information Service, and research papers written by participating investigators and published in scientific or technical journals. Results have also been made available through EPA/NOAA/NMFS project reviews and workshops attended by project participants, and various governmental (Federal and State), private, and public user groups. The final products will be milestone reports summarizing the findings of the major investigative components of the study.

Objectives of the project are (1) to identify and document the types and extent of biological, chemical and physical alterations of the marine ecosystem associated with Buccaneer Gas and Oil Field, (2) to determine specific pollutants, their quantity and effects, and (3) to develop the capability to describe and predict fate and effects of Buccaneer Gas and Oil Field contaminants. The project uses historical and new data and includes investigations both in the field and in the laboratory. A brief Pilot Study was conducted in the autumn and winter of 1975-76, followed by an extensive biological/chemical/physical survey in 1976-77 comparing the Buccaneer Gas and Oil Field area with adjacent undeveloped or control areas. In 1977-78, investigations were intensified within Buccaneer Gas and Oil Field, comparing conditions around production platforms, which release various effluents including produced brine, with those around satellite structures (well jackets) which release no effluents. In 1978-79, studies around Buccaneer Gas and Oil Field structures focused on (1) concentrations and effects of pollutants in major components of

the marine ecosystem, including seawater, surficial sediments, suspended particulate matter, fouling community, bacterial community, and fishes and macro-crustaceans, (2) effects of circulation dynamics and hydrography on distribution of pollutants, and (3) mathematical modeling to describe and predict sources, fate and effects of pollutants. The final year, 1979-80, of study is continuing to focus on items (1) and (2) and on preparation of the milestone reports which will represent the final products of this study.

This project has provided a unique opportunity for a multi-year investigation of effects of chronic, low-level contamination of a marine ecosystem associated with gas and oil production in a long-established field. In many respects, it represents a pioneering effort. It has been made possible through the cooperation of government agencies, Shell Oil Company (which owns and operates the field) and various contractors including universities and private companies. It is anticipated that the results of this project will impact in a significant way on future decisions regarding operations of gas and oil fields on the OCS.

Charles W. Caillouet, Project Manager
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and
William B. Jackson and E. Peter Wilkens,
Editors

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# INTRODUCTION

## Location of Study Area

The area selected for study is the operational Buccaneer Gas and Oil Field located approximately 49.6 kilometers (26.8 nautical miles) south southeast of the Galveston Sea Buoy off Galveston, Texas (Figure 1). This field was selected in 1975 as the study area because: (a) the field had been in production for about 15 years, which time had allowed full development of the associated marine communities; (b) it was isolated from other fields which facilitated the selection of an unaltered area (for comparison) within a reasonable distance of the field; (c) it produced both gas and oil that represented sources of pollutants from marine petroleum extraction; (d) its location simplified logistics and reduced the cost of the research; and (e) the Texas offshore area had not been fully developed for gas and oil production but was expected to experience accelerated exploitation in the future.

## Operation History of Buccaneer Field

Buccaneer Field was developed by Shell Oil Company in four offshore blocks leased in 1960 and 1968 as follows:

| Year | Lease Number | Block Number | Acreage | Hectares |
|---|---|---|---|---|
| 1960 | G0709 | 288 | 2,790 | 1,129 |
| 1960 | G0713 | 295 | 4,770 | 1,930 |
| 1960 | G0714 | 296 | 4,501 | 1,821 |
| 1968 | G1783 | 289 | 2,610 | 1,056 |

In development of the field, 17 structures were built; two are production platforms, two are quarters platforms, and 13 are satellite structures surrounding well jackets. Initial exploratory drilling began about mid-summer of 1960 with mobile drilling rigs. When (as the result of the exploratory drilling) proper locations for platforms were selected, the permanent production platforms were constructed.

There have been no reports of major oil spills from this field. There have been some reported losses of oil due to occasional mechanical failure of various pieces of equipment. The largest

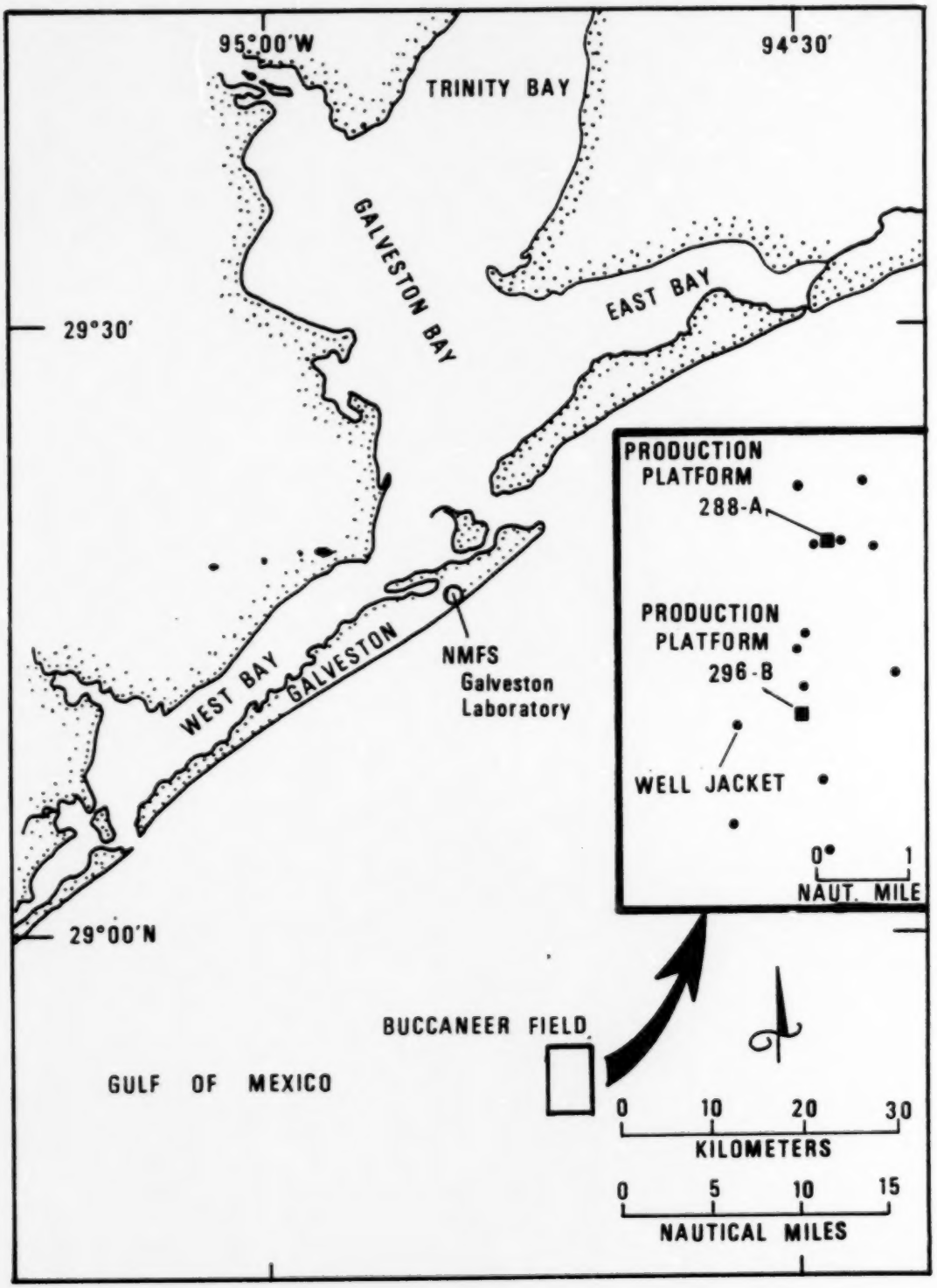


FIGURE 1. LOCATION OF BUCCANEER FIELD

reported spill was three barrels in 1973. The reported oil spill chronology and quantity for Buccaneer Field is as follows:

| Date | Source | Amount | |
|---|---|---|---|
| | | Barrels | Liters |
| September 1973 | Platform 296-B | 0.5 | 79 |
| November 1973 | Unknown | 3.0 | 477 |
| July 1974 | Platform 296-B | 0.5 | 79 |
| August 1974 | Platform 296-B | 1.7 | 265 |
| September 1975 | Platform 288-A | 0.2-0.4 | 38-56 |
| Totals | | 5.9-6.1 | 938-956 |

Buccaneer Field first began operations with the production of oil. Later, when significant quantities of gas were found, the field began producing both oil and gas and has continued to do so to date.

The production platforms and satellites (well jackets) are connected by a number of pipelines with a 50.8 centimeters (20-inch) diameter main pipeline connecting the field to shore. All of the pipelines that are 25.4 centimeters (10 inches) or greater in diameter are buried. The Blue Dolphin Pipeline Company was granted a pipeline permit (No. G1381, Blocks 288 and 296) in 1965 and has operated the pipeline since its construction.

Buccaneer Field occupies a limited area (about 59.3 $km^2$; 22.9 sq. statute miles) leased in the northwestern Gulf of Mexico. Four types of structures are located in Buccaneer Field: production platforms, quarters platforms, satellites (well jackets), and flare stacks. These are shown in Figure 2, which is an oblique aerial photograph of production platform 288-A and vicinity within Buccaneer Field. A map of Buccaneer Field, (Figure 3) depicts the locations of platforms and satellites within the field.

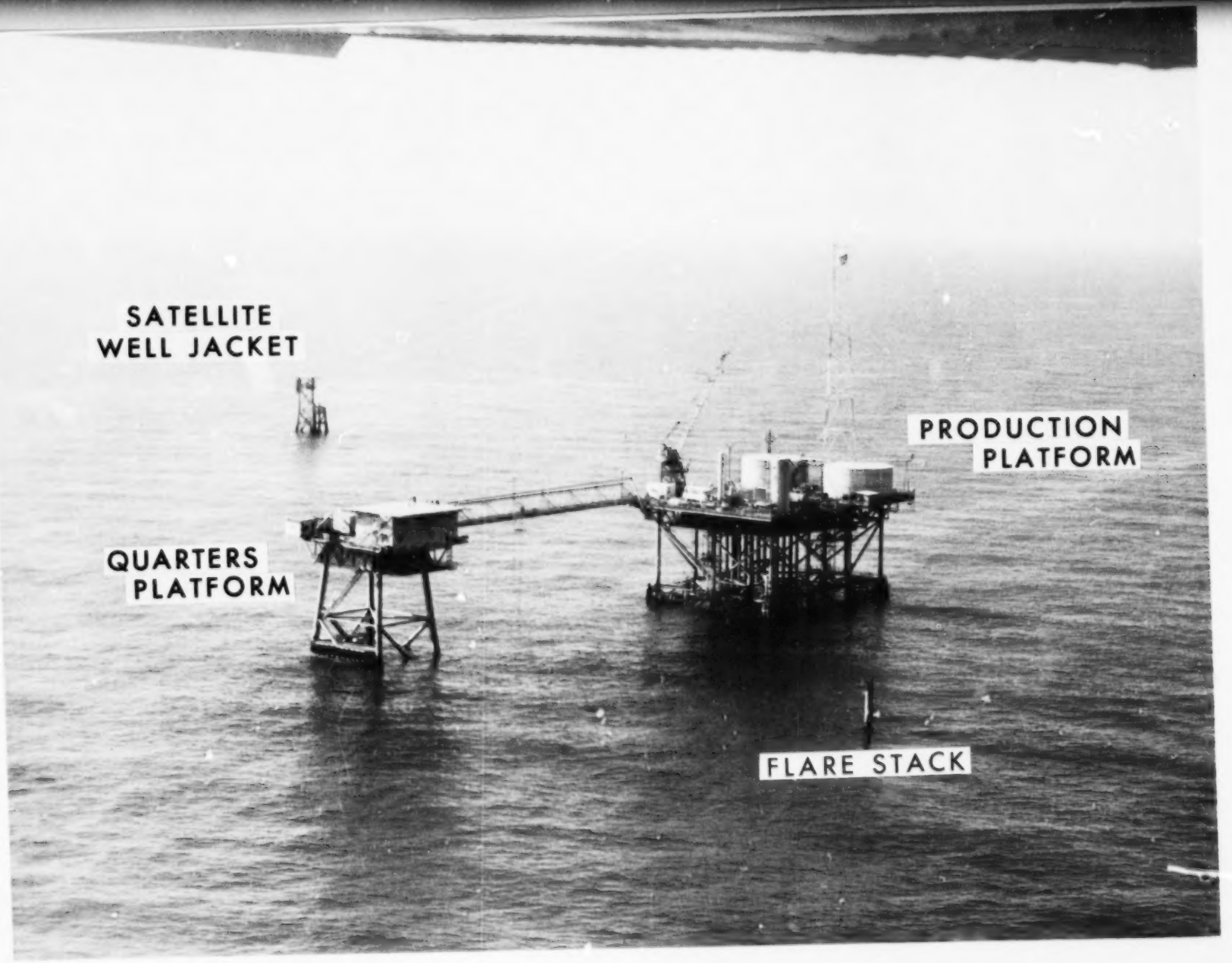


FIGURE 2. BUCCANEER FIELD STRUCTURES

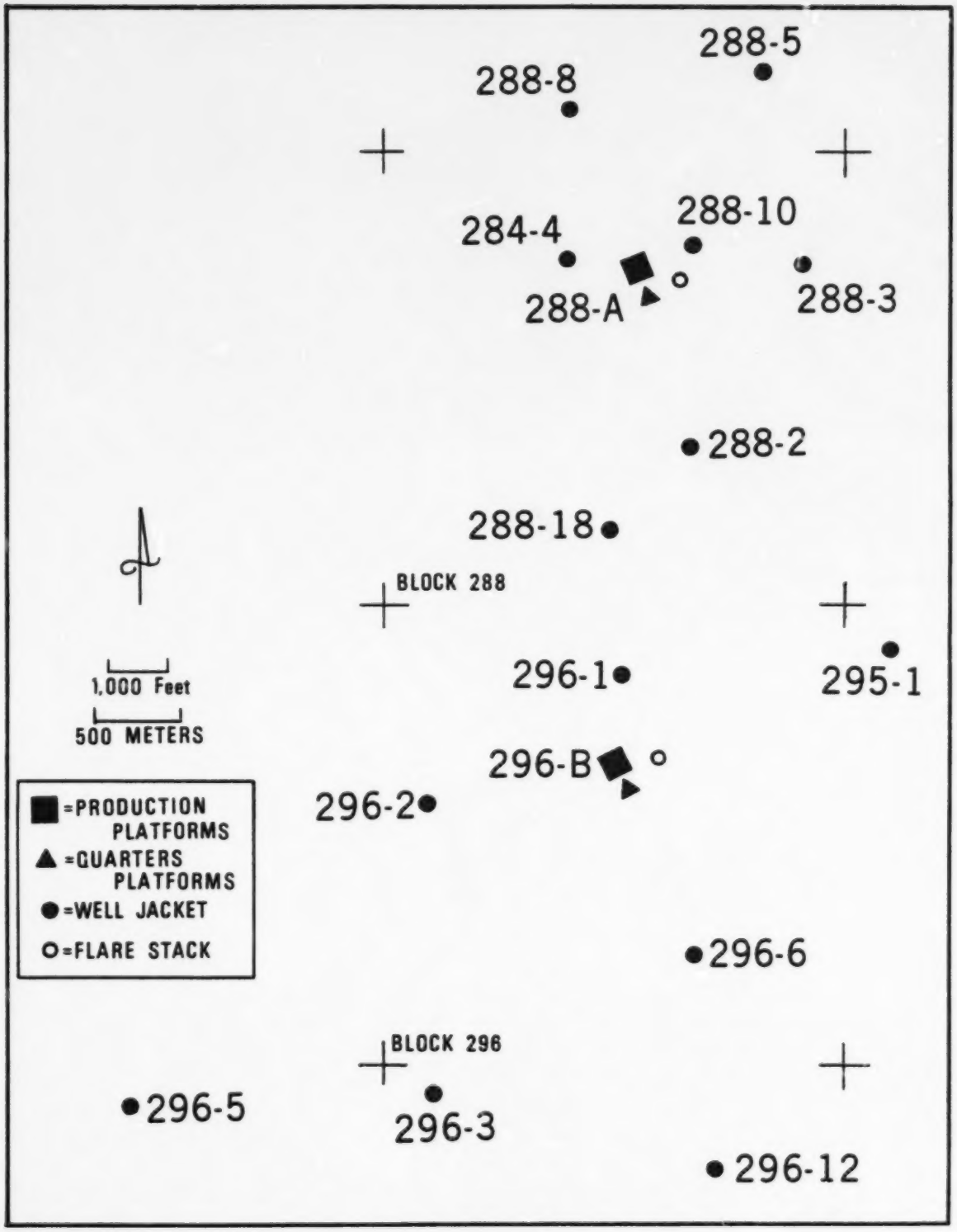


FIGURE 3. SHELL OIL COMPANY'S ALPHANUMERICAL IDENTIFICATION OF BUCCANEER GAS AND OIL FIELD STRUCTURES

# WORK UNIT 2.3.7 - BACTERIAL COMMUNITIES

University of Houston

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## ABSTRACT

A second year study (1978-1979) of the bacterial population of the Buccaneer oil field has been completed. Improved techniques and redirected emphasis were used to confirm or expand upon observations made during the preliminary year of study.

Bacterial numbers and taxonomic types were found to be the same in the oil field as in the control area. No difference in bacterial diversity or biomass was noted between stations but a change did occur between seasonal samples. Members of the genera Vibrio, Pseudomonas, Aeromonas and Acinetobacter were found to predominate in the sediment and fish samples. Vibrio sp. were found to be commonly associated with healthy and some diseased fish. Aeromonas hydrophilia was the only microorganism consistently associated with the diseased fish collected in the oil field.

The bacterial population of the oil field sites contained more oil degrading and sulfur oxidizing bacteria than the control site. Sulfate reducing bacteria were especially abundant in the sediment of the platform sites. The oil field isolates were able to grow readily in Buccaneer brine discharge and some isolates were stimulated by high concentrations of the brine discharge. Both mixed and some pure cultures of Buccaneer field bacteria were able to degrade the alkane portion of Buccaneer condensate. Combined with weathering, some bacterial culture was found to degrade the majority of the alkanes under laboratory conditions in a ten day period.

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## Introduction

Microorganisms, particularly bacteria, constitute the largest numerical population in most aquatic environments and are often the most metabolically active aquatic organisms. The short generation time and great physiological capacity of bacteria as a group enable them to survive and flourish in environments that are inhospitable to other organisms. Indeed, bacterial activity can alter an aquatic environment, thereby affecting the ability of the environment to maintain nonmicrobial populations. Often bacterial activity renders recalcitrant materials more palatable or less noxious, thus improving the environment for other organisms. Petroleum, in general, represents a form of organic pollution that may have adverse effects on aquatic habitats. In the marine environment, microorganisms are the major biotic factors responsible for recycling petroleum (LaRock and Severance, 1973; Walker and Colwell, 1974). Therefore, any comprehensive study of the effects of petroleum on the aquatic biosphere should include a study of bacterial adaptation to petroleum pollution.

Petroleum and associated pollutants have a number of effects on the microbial population. Some components of oil are recalcitrant and may be inhibitory to some portions of the bacterial population (Atlas, 1975, Walker and Colwell, 1974). One of the objectives of this study was to determine the effects of oil pollutants on the normal bacterial population of uncontaminated sites and to estimate the success of the microbial population around the production platforms to cope with the petroleum .

Microorganisms are not passive individuals which merely respond to habitat changes, but are actively altering the aquatic environment. Estimations of the rate of attack on petroleum products by bacteria combined

with an appreciation of the by-products resulting from microbial degradation are vital to determine the effect of oil pollution in the marine environment. This information can be used to estimate the fate of petroleum products in the ocean and therefore, is vital in assessing the impact of oil field contaminants. To date, bacteriologists have concentrated a large portion of their studies on unexpected large oil spills occurring near coastlines (Kerr, 1977). These acutely oil-polluted sites have been found to support relatively large bacterial populations containing an abnormally high proportion of bacteria capable of degrading oil. Production platforms in the Gulf of Mexico afford a unique opportunity to study the microbial ecology of oil degradation because they represent an area of long-term exposure to low levels of oil pollutants. These sites of chronic oil contamination may be the most severely damaged environments (Loughry, 1977), but the autochthonous bacterial population of these areas has had a maximal opportunity to adapt to the presence of the platforms. This study was designed to determine the extent of microbial adaptation around the oil drilling site.

The first year's study (1977-78) of the bacteria in the Buccaneer oil field showed that dramatic differences did not exist between the oil field and the control area. The second year's study (1978-79) was designed to insure that any subtle differences in population in the two sites could be observed. The specific objectives of the second year of study were: (1) to observe subtle effects of the production platform on bacterial numbers in the water, sediment and suspended particulate material; (2) to examine the taxonomic composition of the bacteria found in sediment samples from the field and from the control area; (3) to investigate the bacteria associated with diseased fish in the study area; (4) to determine

the capacity of the bacteria from the field to utilize oil and its potential for oxidizing sulfur; and (5) to observe any detrimental effects that Buccaneer field brine discharge might have on bacteria.

## Methods and Materials

Samples of water for this study were collected with Niskin sterile bag samplers. Sterile glass bottles were used to collect surface water samples. Surface sediment samples were collected with a Petite Ponar Grab. Water and sediment were always subjected to bacteriological analysis immediately after collection. (Suspended particulate material was collected in the water samples by the Niskin sampler). The sewage effluent from the platform was not sampled directly because of difficulties in maneuvering the ship close to the rig. Therefore, samples were collected as close as feasible to the effluent pipe of the living quarters platform and examined carefully for traces of dilute sewage effluent. Brine discharge was studied by examining samples collected on the rigs and returned to the laboratory.

Samples from 10 sites were routinely collected. These areas include sites as near as possible to platform 288-A, platform 296-B, flare stack 288-10, well jacket 288-8 and the living quarters of platform 296-B. Multiple samples (3) were collected at platforms 288-A and 296-B in an attempt to identify a gradient around these two platforms (see Figure 1). These sites were sampled at regular intervals starting at the platform and extending approximately 300 feet down current. The exact location of the site was determined by the capacity of the research vessel to approach the platform and the currents. A control station five miles north of the study area was also sampled.

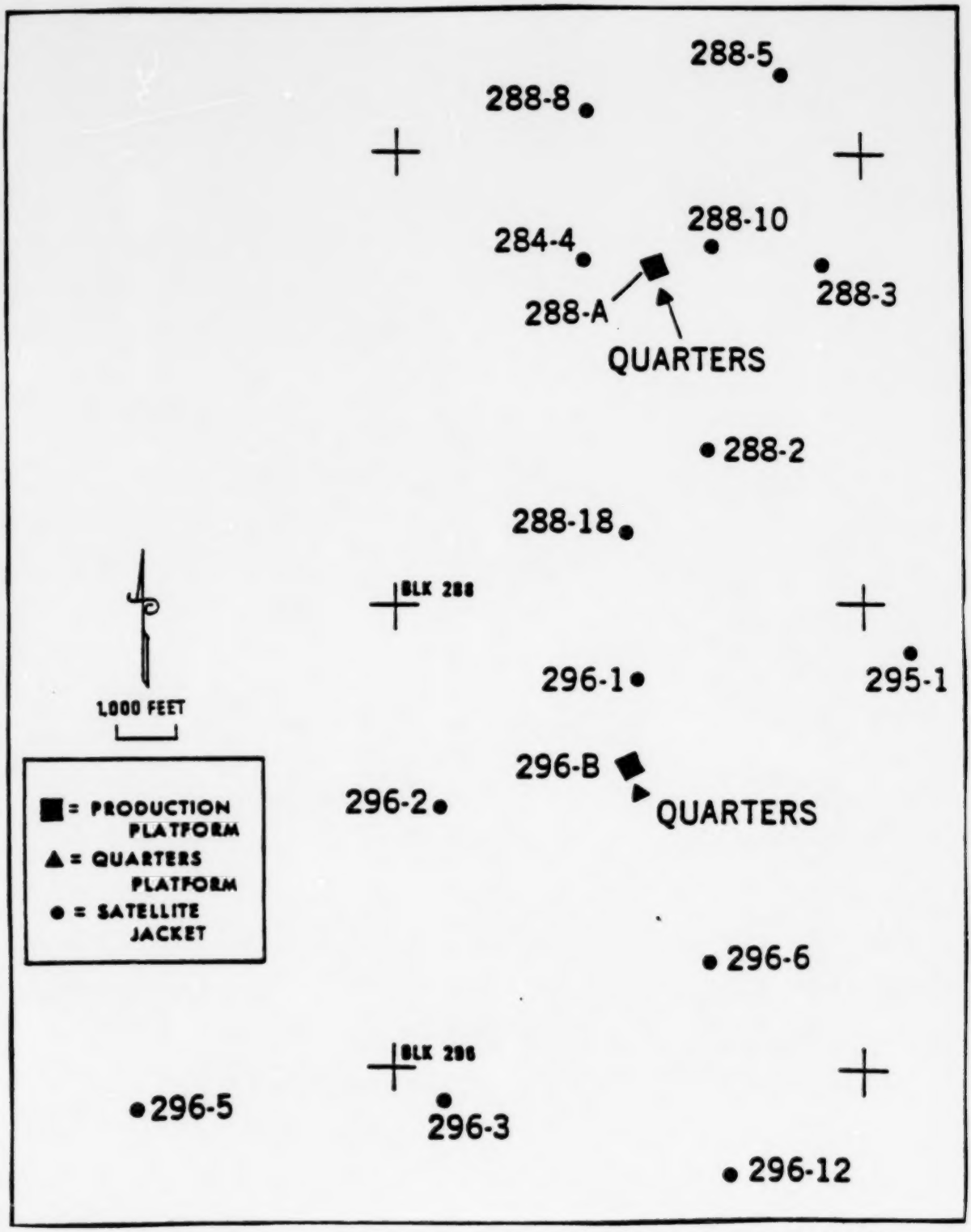


Figure 1. Platform designations and locations in the Buccaneer oil field.

Initial bacterial enumerations (colony forming units - CFU) were made on MSWYE medium which is composed of 1 g proteose peptone and 1 g yeast extract in 1000 ml of 3 salt solutions (0.4M NaCl, 0.028 M $MgSO_4 \cdot 7H_2O$, 0.01 M KCl). The medium was brought to pH of 7.2-7.4 with 1N NaOH. MSWYE agar was prepared by the addition of 20 grams of Bacto agar. This medium was used to collect a representative set of microorganisms for laboratory analysis. Each sample was divided into 4 subsamples, each of which was plated in duplicate. Other shipboard analyses included inoculation of broth tubes for the determination of the number of hydrocarbon-utilizing and autotrophic sulfur-oxidizing bacteria. Oxidation of sulfur was detected by a change in the color of the medium pH indicator as the acidic products of sulfur oxidation accumulate. Hydrocarbon degradation was tested in a two step procedure. Initially the samples and dilutions of the samples were placed in an inorganic salts plus a crude oil medium. The crude oil used was from the Buccaneer field. Confirmation of hydrocarbon utilization was made by transferring some of the presumptive positive strains from oil medium to silica gel oil media or to a second passage in the oil media. Salt-requiring bacteria were enumerated by plating water samples on MSWYE-like medium made with distilled water instead of the salt solution.

The water around the sewage discharge was examined for the presence of fecal coliforms. This screening involved presumptive testing by plating on MacConkey agar followed by examination of the positive strains for taxonomic characteristics typical of coliforms.

Laboratory based analysis included bacterial numbers and biomass estimates determined by epifluorescent microscopy (Hobbie, et al, 1977). Water samples were fixed with formalin on board ship immediately after

collection. The fixed cells were stained with Acridine Orange and counted with an Olympus BH-B epifluorescent microscope equipped with oil immersion optics. For each sample, 25 microscopic fields (with 10-30 bacteria/field) were examined. Bacterial cell volumes were estimated to determine bacterial biomass. Water samples were returned to the laboratory for determination of bacterial numbers attached to suspended particulate material. Sterile Millipore filters of different pore sizes were used to remove different fractions of the suspended materials. Bacterial counts using MSWYE agar were performed on each filter fraction and the number of bacteria remaining was compared to the initial total viable count.

The makeup of the bacterial community was determined by taxonomic analysis of 511 isolates collected from MSWYE plates. A minimum of 50 strains from the control site and 50 strains from the sampling site closest to platform 296-B were collected during each cruise. The strains were identified to a genera level since the primitive state of marine bacterial taxonomy makes species designation ambiguous. Taxonomic tests which were run on the strains included: gram stain, Kovac's oxidase test, M.O.F. reactions, flagella stain, catalase reaction, spore stain, 0/129 sensitivity and arginine dihydrolyase. Other tests were included when necessary. All the stains were also tested for oil degrading, sulfur oxidizing and sulfur producing potential. A simple taxonomic key designed specifically for marine bacteria was used to identify the strains (Holloway, et al., 1980).

One hundred and nine bacterial strains were collected from diseased fish and bioassay samples (from Work Unit 2.3.4 and 2.3.5). The isolates which appeared to be the predominant colony type of swab plates derived from the samples were chosen for testing on A.P.I. diagnosis strips. The key provided with the strips was not utilized since it is biased toward human pathogens. Instead the large number of taxonomic tests provided by the strips were used in keys (e.g. Bergey's Manual of Determinative Bacteriology).

Bacterial biodegradation experiments used mixed and pure cultures collected in the field. Mixed culture inocula were freshly collected water samples or were prepared by diluting sediment in sterile sea water, centrifuging to pellet the sediment, and using the supernatant as a mixed culture inoculum. Mixed culture experiments were used to give an indication of the turnover rates of petroleum in the environment. Unforunately, mixed culture is a complex interaction of bacteria that a bacteriologist cannot fully control. Therefore, degradation rates for individual bacteria are vital to the calculation of maximal turnover rate and to assign roles in petroleum degradation to various components of the microbial population. The pure culture experiments used strains identified during the taxonomic portion of the study. These included isolates from Platform 296-B and the control area, as well as laboratory control microorganisms (e.g. E. coli and an oil-degrading Acinetobacter). Some of the strains which appeared to degrade petroleum upon initial isolation were examined in detail. To increase the probability of meaningful data, all degradation experiments used the same initial pH, salinity, and approximate temperature as the sampling sites. Experiments were run for time periods ranging from 3 to 30 days with some subsampling at intermediate times. The bacterial numbers in the inoculum were manipulated to resemble water column total viable counts. The degradation experiments were run in sterile artificial sea water which had been enriched with $PO_4$ and $NO_3$. Hydrocarbons were introduced by adding either filter sterilized Buccaneer condensate or filter sterilized brine discharge (either at full strength or diluted). The degradation rates of various hydrocarbon components were calculated by careful measurement of the hydrocarbon components of the degradation experiment and comparing them with a sterile control prepared and incubated

the same time as the experimental flask. Estimates were made of the total portion of the hydrocarbons utilized as well as measurements of the loss of some of the specific components of the hydrocarbon mixture.

Qualitative and quantitative measurements of the hydrocarbons were made using an Antek 300 dual column temperature programmable gas chromatograph equipped with glass packed and capillary columns. The extraction, fractionation and concentration techniques used were essentially those proposed by Dr. Brian Middleditch in his study of the hydrocarbons of Buccaneer field. This procedure calls for extraction of an acidified sample using equal volumes of cyclohexane. The sample is then fractionated into "alkane", "aromatic", and "asphaltene" components using various solvents on a silica gel column. The resulting fractions are concentrated under nitrogen and stored in the dark until gas chromatographic analysis. Deuterated hydrocarbon standards of known concentration are added to the sample before extraction for use as an internal standard in quantitation. The emphasis in the degradation experiments was on the degradation of the aliphatic alkane components since we were more confident in our identification of these components.

The effect of Buccaneer field discharge was tested by growing pure and mixed cultures of Buccaneer field isolates as well as laboratory isolates in the presence of various concentrations of brine. Either 1% or 90% solutions of refrigerated filter sterilized brine discharge in sterile 3 salts solution were used. Pure cultures used in this study included isolates taken from the taxonomic analysis or laboratory control isolates. Mixed cultures were aliquots of freshly collected Buccaneer field water samples. At varying time intervals the growth flasks were sampled and the bacterial numbers determined by plating on MSWYE agar. A few experiments were monitored for sulfur loss and pH shift in the growth flask.

## Results

Bacterial counts were successfully made for 10 stations during the 4 seasonal cruises. Both enumeration techniques (total viable count and eipfluorescent) were used. The bacterial counts in the water samples ranged from 63 to 1,600 per ml as counted by plating, and from 73,100 to 1,830,000 per ml when counted by epifluorescent microscopy. The range of bacterial counts in sediment samples was 491,000 to 17,200,000 per gram dry weight of sediment. All sediment samples were enumerated by plating. No differences were noted betwee1 the total bacterial numbers found at different stations or during 3 of the 4 seasonal cruises (summer, winter, and spring). The fall cruise (November 2-3, 1978) showed 10 fold lower bacterial counts in the water samples. The sediment samples from the fall cruise were within the range reported for the other 3 cruises. Table 1 gives the ranges of bacterial counts obtained by both enumeration techniques during this study.

Epifluorescent microscopy proved to be an excellent technique for determining direct bacteria counts in the water samples. In general, the epifluorescent counts were 2,000 to 3,000 times higher than counts obtained by plating techniques. Furthermore, the same general distribution pattern obtained by plating enumeration was seen with the epifluorescent counts. Vertical profiles in the bacterial numbers of the water column were observed with both techniques. During Cruises I (August 1978) and II (November 1978) the bacteria numbers were consistently highest in the bottom water samples and lowest in the surface samples. This pattern was observed repeatedly by both enumeration

Table 1

Range of Bacterial Counts

A. Total Viable Counts

| Cruise | Date | Source | Lowest | Highest |
|---|---|---|---|---|
| I | August | Water | $2.2 \times 10^{2}_{5}$ | $1.1 \times 10^{3}_{7}$ |
| | | Sediment | $4.9 \times 10^{5}$ | $1.2 \times 10^{7}$ |
| II | November | Water | $6.3 \times 10^{1}_{6}$ | $3.73 \times 10^{2}$ |
| | | Sediment | $1.9 \times 10^{6}$ | $6.0 \times 10^{6}$ |
| III | February | Water | $1.4 \times 10^{2}_{5}$ | $1.6 \times 10^{3}_{7}$ |
| | | Sediment | $8.1 \times 10^{5}$ | $1.7 \times 10^{7}$ |
| IV | May | Water | $2.6 \times 10^{2}_{5}$ | $9.4 \times 10^{2}_{6}$ |
| | | Sediment | $6.0 \times 10^{5}$ | $8.6 \times 10^{6}$ |

B. Epifluorescent Counts

| | | | | |
|---|---|---|---|---|
| I | August | Water | $7.3 \times 10^{4}$ | $3.0 \times 10^{5}$ |
| II | November | Water | $1.3 \times 10^{5}$ | $4.0 \times 10^{5}$ |
| III | February | Water | $1.7 \times 10^{5}$ | $1.8 \times 10^{6}$ |
| IV | May | Water | $3.2 \times 10^{5}$ | $1.3 \times 10^{6}$ |

procedures. Cruise III (February 1979) and Cruise IV (May 1979) did not exhibit the same patterns as the first two cruises. Indeed, many differences were noted between Cruises I and II as compared to II and IV. Bacterial counts for Cruise III were highest in surface water samples and declined in numbers near the bottom. The relationship between epifluorescent data and plating data during Cruise III was not as consistent as the first two cruises. The middle water sample often varied in its relationship to other samples depending on the enumeration technique. Cruise IV water samples showed higher epifluorescent counts in the surface water, but the plating data indicate higher total viable counts in the bottom water sample.

Bacterial biomass estimates were calculated from the data obtained by both enumeration techniques (total viable count and epifluorescence). No differences were noted between the biomass average of the 4 cruises. The epifluorescents technique gave biomass estimates ranging from $6.2 \times 10^{-6}$ to $4.4 \times 10^{-5}$ gC/l which were approximately a thousand fold higher than the biomass estimates made with the total viable count data ($3.9 \times 10^{-9}$ to $6.0 \times 10^{-8}$ gC/l). Since the biomass data reflects the enumeration data, the same patterns discussed concerning the bacterial distribution were observed in the bacterial biomass distribution.

Bacteria in both the control area and the platform site appeared to be attached to similar sized particulate material. Only 20% of the colony forming units were found to be associated with particulate more than 50 μ in diameter. Ten percent of the bacterial population (CFU) appears to be attached to particulates between the size of 8 μ and 3 μ, and another 10% are either free living or associated with very small

(<3 μ) particulates. The remaining 60% of the bacterial (CFU) population appears to be associated with particulate material between the size of 8 μ and 50 μ. Epifluorescent observations were subjective, but it appeared that most of the bacteria in the epifluorescent samples were free living or existed as mini-colonies (3 or 4 cells clumped together).

Several physiological capacities of the bacterial population were sampled. Salt requiring bacteria were found to predominate in the samples examined. Ninety-four percent of the total viable bacteria in the study area and control site required salt for growth. This percentage was higher in the sediment (97% average) than the water column samples (93% average). More salt requiring bacteria were found during Cruises III and IV (averaging 95% and 97% respectively) than Cruises I and II (averaging 91% and 92%).

Oil degrading bacteria were found to be more common in samples collected in the oil field as compared to the control area (Table 2). The highest percentage of oil degrading bacteria was found in the top water samples near platform 296-B, and , in general, the top water samples contained higher percentages of oil degrading bacteria than the middle or bottom samples. The number of oil degrading bacteria decreased rapidly with distance from the platform in the water column, but in the sediment samples the decrease was less obvious. The percentage of oil degraders in the sediment at well jacket 288-8 proved to be as high as any sample examined. Flare stack 288-10 contained oil degrading bacteria in numbers roughly equivalent to samples collected equidistant on the other side of the platform. The sediment strains collected for taxonomic examination were also screened for oil degrading capacity. Table 3 shows that the

Table 2

Average Percent Oil Degrading Bacteria*

| Station | Water Column | | | Sediment | Average |
|---|---|---|---|---|---|
| | Top | Middle | Bottom | | |
| Well Jacket 288-8 | 0 | 0.07 | 0.18 | 0.82 | 0.27 |
| Platform 288-A | .38 | 0.005 | 0.24 | 0.52 | 0.29 |
| Flare Stack 288-10 | .485 | .21 | .16 | .025 | .22 |
| Platform 296-B | .95 | .21 | .23 | .17 | .39 |
| Plat. 296-B + 100 ft. | .225 | .078 | .26 | .26 | .21 |
| Plat. 296-B + 200 ft. | .073 | .23 | .12 | .23 | .16 |
| Control | .07 | .013 | .098 | .098 | .069 |

*Represents average of four cruises

Table 3

Physiological Capacity of Taxonomic Isolates

Platform 296-B

| Cruise | % oil degraders | % sulfur oxidizers | % sulfate reducers |
|---|---|---|---|
| I | 0 | 7 | 18 |
| II | 0 | 4 | 21 |
| III | 9 | 4 | 18 |
| IV | 9 | 6 | 36 |

Control Site

| Cruise | % oil degraders | % sulfur oxidizers | % sulfate reducers |
|---|---|---|---|
| I | 0 | 0 | 3 |
| II | 0 | 0 | 8 |
| III | 1 | 3 | 6 |
| IV | 1 | 1 | 13 |

highest number of isolates capable of utilizing oil is from the platform areas.

Sulfur metabolizing bacteria were more common in the oil field than the control area (Tables 3 and 4). Autotrophic sulfur oxidizing bacteria were most abundant in the top water samples taken near platform 296-B and flare stack 288-10. A higher percentage of autotrophic sulfur oxidizing bacteria were found in the water samples than in the sediment samples. Sulfur oxidation was also a test used on the sediment strains used for taxonomic analysis (Table 3). A higher percentage of these isolates showed sulfur oxidizing potential than would be indicated by the autotrophic sulfur oxidizing counts (Table 4). A higher percentage of the oil field isolates, used in the taxonomic tests, showed sulfur oxidizing potential than the control site isolates. These taxonomic strains were also examined for sulfate reducing capacity, and a dramatically higher percentage of the platform isolates were positive as compared to the control site isolates (Table 3).

A total of 532 sediment isolates, half from the control area and half from platform 296-B were identified to genera level. A summary of the various genera found during the study is given in Table 5. The predominate genera were Vibrio, Pseudomonas, Aeromonas, and Acinetobacter, respectively. A Shannon Weiner diversity index was calculated for each sampling site and each cruise (Table 5). Essentially, no difference was observed between the control site and the platform sites, but there was a dramatic decrease in diversity from Cruise I to Cruise IV. The decreased diversity observed during Cruise IV results largely from the increased number of Vibrio sp. found.

Table 4

Average Percent Sulfur Oxidizing Bacteria*

| Station | Water Column | | | Sediment | Average |
|---|---|---|---|---|---|
| | Top | Middle | Bottom | | |
| Well Jacket 288-8 | .23 | .02 | .29 | .01 | .13 |
| Platform 288-A | .46 | .02 | .43 | .08 | .25 |
| Flare Stack 288-10 | 5.29 | .63 | .33 | .06 | 1.58 |
| Platform 296-B | 6.32 | 2.72 | 1.19 | .01 | 2.56 |
| Plat. 296-B + 100 ft. | .23 | .13 | .16 | .03 | .14 |
| Plat. 296-B + 200 ft. | .21 | .38 | .75 | .08 | .34 |
| Control | .78 | .35 | 1.20 | .06 | .60 |

*Represents average of four cruises

Table 5

Taxonomic Composition of Isolates

| Taxon | Cruise I - August | | Cruise II - Nov. | | Cruise III - Feb. | | Cruise IV - May | |
|---|---|---|---|---|---|---|---|---|
| | Platform | Control | Platform | Control | Platform | Control | Platform | Control |
| Aeromonas | 8 | 9 | 12 | 12 | 6 | 21 | 8 | 12 |
| Acinetobacter | 2 | 1 | 0 | 1 | 0 | 4 | 0 | 2 |
| Acinetobacter lwoffi | 6 | 11 | 3 | 11 | 6 | 3 | 0 | 2 |
| Bacillus | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Coryneforms | 5 | 3 | 3 | 1 | 1 | 0 | 1 | 0 |
| Cytophaga | 5 | 4 | 3 | 6 | 1 | 0 | 0 | 0 |
| Enterobacteriaceae | 2 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Flavobacterium | 3 | 4 | 2 | 5 | 0 | 0 | 0 | 0 |
| Flexibacter | 3 | 2 | 3 | 4 | 3 | 1 | 0 | 0 |
| Lactobacillus | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Lucibacterium | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 |
| Micrococcus | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 |
| Moraxella | 3 | 3 | 2 | 6 | 3 | 1 | 2 | 0 |
| Planococcus | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Pseudomonas | 4 | 3 | 2 | 6 | 8 | 7 | 0 | 3 |
| Pseudomonas Gp 2 | 0 | 4 | 0 | 0 | 5 | 0 | 1 | 0 |
| Pseudomonas Gp 3 | 8 | 11 | 12 | 8 | 8 | 7 | 8 | 7 |
| Vibrio | 12 | 4 | 23 | 5 | 23 | 24 | 45 | 40 |
| Diversity (H'=) | 2.45 | 2.44 | 2.14 | 2.23 | 2.01 | 1.63 | 1.11 | 1.33 |

A total of 80 fish (76 healthy and 4 diseased) and 16 shrimp (previously used in work unit 2.3 bioassays) were examined for potential bacterial pathogens. The taxonomic makeup of the bacteria found on the fish and shrimp was similar to the sediment isolates, with members of the genera Vibrio, Pseudomonas, Aeromonas, and Acinetobacter predominating. Only one bacterium Aeromonas hydrophila was found associated with all the diseased fish. Other reported fish pathogens, including Vibrio alginolyticus, were found associated with apparently healthy fish.

Water and sediment samples from each of the four cruises were examined for the presence of fecal coliforms. Presumptive coliform organisms were found commonly (up to 195/ml water and $5.2 \times 10^5$/gram sediment) during each cruise and were usually higher at the crew quarters station than the control site. However, when these presumptive isolates were examined in greater detail, no fecal coliform microorganisms were found in any samples.

Brine discharge from Buccaneer oil field did not prove to be bactericidal. All the mixed culture inocula and the pure culture showed growth in dilute and undiluted (90% discharge in sterile seawater) brine discharge. This includes mixed cultures from both the platform area and the control area. Some of the pure platform area cultures actually grew faster in the higher concentration of brine discharge. The non-oil field control isolates did not grow readily in the brine discharge and appeared to be reduced in number after short term exposure to the brine discharge.

Nine biodegradation experiments were completed using eleven oil field isolates, twenty-seven mixed cultures, and three control strains. The experiments were run using different time intervals ranging from 3 days to 30 days incubation periods in order to assess the oil

degrading capabilities of BOF (Buccaneer Oil Field) bacteria.

Only the "alkane" fractions were reported, although other fractions were saved for subsequent analysis. An example of a hydrocarbon "alkane" fraction at the start of the biodegradation experiments is shown in Figure 1. A weathered, uninoculated control incubated for the same period of time as the biodegraded sample was used as a base line to estimate the actual amount of bacterial activity. By this technique, the amount of abiotic hydrocarbon loss can be subtracted from the bacterial hydrocarbon degradation.

Three hydrocarbon sources were utilized during this year's study--Buccaneer crude oil, brine discharge, and diluted brine discharge. The low levels of alkanes present in the brine and diluted brine discharge experiments posed a difficult problem for analysis of biodegradation. Therefore, generalizations are difficult to make concerning comparisons between different bacterial types (sediment vs. water bacteria) and different locations (Oil field area vs. control area bacteria).

In this study, many BOF isolates did seem to show some biodegradative abilities towards BOF oil and brine discharge. Alkanes showed the typical biodegradation patterns as seen in last year's work and documented in literature . The first group of alkanes, $C_{12}$-$C_{20}$, are the most rapidly susceptible to bacterial degradation. The second group, $C_{21}$-$C_{30}$, are utilized at much slower rates. The group $C_{31}$-$C_{36}$ are utilized very slowly in comparison with other groups.

Abiotic loss (weathering) also showed similar trends with $C_{12}$ and $C_{13}$ lost very rapidly to evaporation; $C_{14}$-$C_{30}$ weathered at slower rates; and $C_{31}$-$C_{36}$ lost at very slow rates.

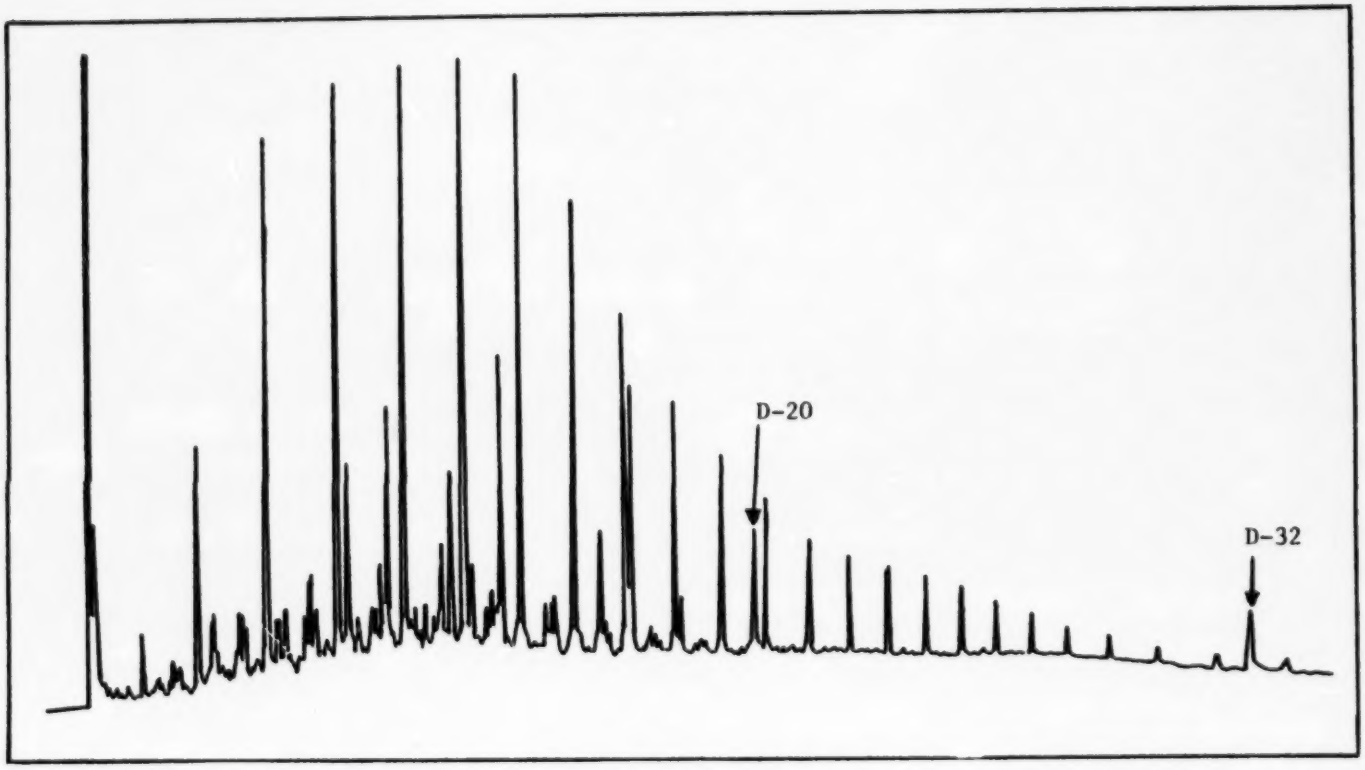


FIGURE 1. GAS-LIQUID CHROMATOGRAPHIC TRACING OF "ALKANE" FRACTION OF BIODEGRADATION GROWTH MIXTURE (INORGANIC SALTS SOLUTION + 0.1% BUCCANEER CONDENSATE) BEFORE INOCULATION. D-20 AND D-32 REPRESENT DEUTERATED C-20 AND C-32 ADDED AS INTERNAL STANDARDS AT A CONCENTRATION OF 2.5 PPM.

One culture (B-13) isolated from the oil field appeared to degrade 55% of the total alkanes ($C_{12}$-$C_{36}$) in brine discharge within a ten day period. In this experiment, hydrocarbon loss was also observed for the higher alkanes, $C_{20}$-$C_{28}$ (60% of the total). It should be noted , however that qualitative analysis of hydrocarbon loss due to bacterial degradation is difficult to accurately measure with increasing hydrocarbon chain length and decreasing quantities of hydrocarbon components.

Mixed cultures showed a significant amount of biodegradation of BOF crude oil. In one large experiment, mixed sediment cultures appeared to degrade 60% of the total alkanes with specific components being degraded at up to 80% within a 28 day incubation period (Fig. 2). These components included the higher hydrocarbons, $C_{15}$-$C_{28}$, and also the more difficult to degrade branched hydrocarbons, Pristane and Phytane. Differences in biodegradative abilities between oil field and control sediment bacteria were observed with some increased effectiveness in hydrocarbon utilization being seen in the oil field sediment bacteria.

Water column bacteria were also found to degrade alkane hydrocarbons but at a reduced rate. After a 14 day incubation period, 25% of the total alkanes appeared to be lost due to biodegradation. There seemed to be a more preferential treatment of hydrocarbon utilization than that seen in the sediment bacteria with branched alkanes being found to persist along with other unidentified components. As with the sediment bacteria, comparisons between degrading abilities of control and oil field mixed cultures from the water column showed only a small difference in effectiveness of the oil field microorganisms.

Seasonal differences in microbial degradation were observed in mixed cultures obtained from both water and sediment samples. Mixed

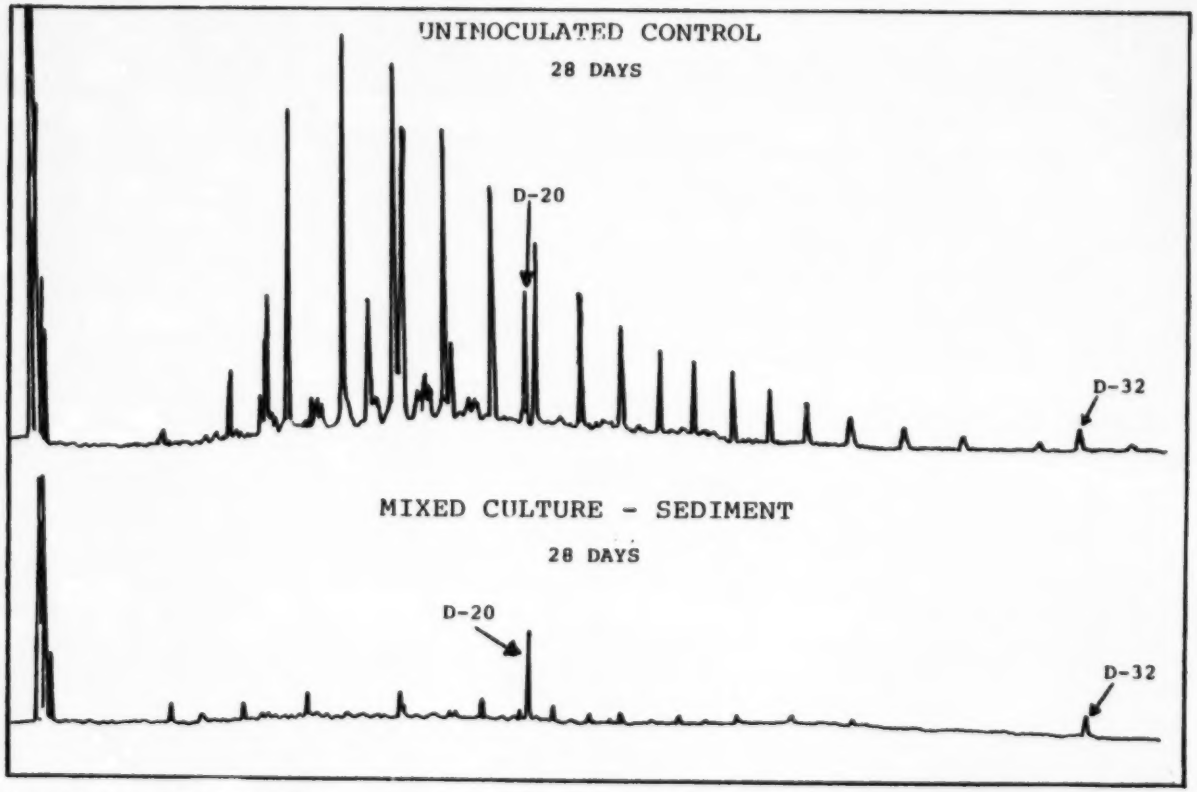


FIGURE 2. GAS-LIQUID CHROMATOGRAPHIC TRACING OF UNINOCULATED WEATHERED CONTROL (TOP) VS. BIODEGRADED SAMPLE (BOTTOM). D-20 AND D-32 ARE DEUTERATED INTERNAL STANDARDS ADDED AT A CONCENTRATION OF 2.5 PPM.

cultures obtained during the winter - fall season appeared to have reduced alkane degrading capacities as compared to the cultures collected during the spring - summer season.

## Discussion

No differences in bacterial numbers, biomass, or taxonomic composition between the platform site and the control area were noted in this study. During the 1977-1978 study a difference was noted in the biomass estimates and bacterial numbers (CFU). In this study improvements were made in the enumeration techniques and subsampling was increased. The improved data indicate that the differences observed previously were a chance occurrence and probably do not represent a consistent pattern.

Although there was not a dramatic difference in the bacterial populations of the sampling site, there was a difference between sampling cruises. The bacteriological data from Cruise I and II is markedly different than that of Cruise III and IV. In general, Cruise I and II showed a lower percentage of salt requiring bacteria, a higher presumptive coliform count, a higher diversity among the bacterial taxa, a higher bacterial count in the bottom water compared to the surface, a lower percentage of oil degrading and sulfur metabolizing bacteria in the sediment and a better correlation between the plating counts and the epiflourescent counts than Cruise III and IV. The differences between these two pairs of cruises is dramatic and is difficult to explain. One phenomenon; the decrease in diversity among the bacterial populations which was observed in Cruise III and especially in Cruise IV, can be explained by the great increase in the prevalence of members of

the genera Vibrio. This bloom of Vibrio sp. is probably related to season and water temperature changes. The other differences observed are probably related to currents. During Cruise I and II surface water currents flowed toward the northeast and during Cruise III and IV the flow was toward the southwest.

The majority of the bacteria in the water column of the platform area and the control site were found to be associated with suspended particulate material. Ninety percent of the total water column bacterial population (CFU) was found to be associated with particles larger than 3μ. The extent of the bacterial association with particulate material is remarkable and deserves a good deal of study to permit an understanding of the microbial ecology of the area. The distribution and movement of bacteria in the water column is probably controlled by the distribution of particulates and the effect of currents on particulates.

Epifluorescent microscopy was used to estimate bacterial numbers during this year's study of the oil field. The resulting bacterial numbers were consistently higher than data generated by plating counts during either last year's or this year's study. The epifluorescent counts generally showed the same distribution pattern as the plating data but gave much higher bacterial numbers. The higher bacterial numbers give a better estimate of bacterial biomass and emphasize the importance of bacterial population in the ecology of the study area.

Oil degrading bacteria were more abundant in the oil field site, especially in the surface water samples,than in the control site. This distribution followed the presumed logical distribution of hydrocarbons (i.e. in the surface water around the brine discharge point). Sediment

isolates capable of degrading oil were also more common in the oil field but the decrease in the percentage of oil degrading strains in samples collected at increasing distance from the platform was not as dramatic as the decrease seen in the water samples. There were similar numbers of oil degrading bacteria in the sediment at well jacket 288-8 and platform 288-A. Therefore, the well jacket 288-8 station which had been suggested as a control site is not sufficiently removed from the platform to be used as a control site, at least in bacteriological studies.

Sulfur oxidizing bacteria were also more common in the oil field than the control area. The largest number of sulfur oxidizing strains found during the enumertion studies were located in the water column. However, the highest percentage of sulfur oxidizing bacteria was found among the sediment isolates used in the taxonomic analysis. These high percentages could be due either to the less stringent nonautotrophic criteria used to isolate and test the taxonomic strains or false positive tests among the taxonomic strains due to media carryover into the sulfur MPN tubes. Several members of the sulfur oxidizing genera, Thiobacillus, are not strictly autotrophic in that they require organic growth factors. These isolates would not be detected by the sulfur MPN tubes used for enumeration. This may account for the high percentage of sulfur oxidizing bacteria seen among the sediment taxonomic isolates which were not initially isolated on a medium selective for autotrophic.

One group of sulfur metabolizing bacteria, the sulfate reducers, were found to be common in the oil field. Only the sediment strains

used in the taxonomic study were checked for sulfate reducing ability. There was a decidedly higher number of sulfate reducers at the platform site compared to the control area. This distribution indirectly indicates that there must be a large amount of sulfur in the form of sulfate around the platform to serve as a nutrient to the sulfate reducing bacteria. The presence of a sulfur driven bacteria system in the platform area is possible and therefore the sulfur effluent from the rig deserves a good deal more study.

The taxonomic analysis of sediment bacteria during this year's study produced data similar to last year's study of water column isolates. Vibrio, Pseudomonas, Aeromonas, and Acinetobacter were the predominate genera in the platform and control site. No difference in the taxonomic diversity of the two sites was noted but a seasonal change in the diversity index of each site was seen. This change in diversity appears to be due to the large increase in Vibrio sp. seen in Cruise III and IV. This increase is due to the seasonality of Vibrio sp. which are warm water bacteria that reside in the sediment in the winter and spring but live mainly in the water column during the warmer months (Kaneko and Colwell, 1973). The dominance of Vibrio sp. in the winter and spring cruises is largely the cause of the apparent change in diversity. The fish isolates showed a similar taxonomic composition to the sediment isolates. One important exception was noted. Several potential fish pathogens (e.g. Vibrio alginolyticus) were detected on apparently healthy fish but only one bacterium, Aeromonas hydrophila, was found associated with the 4 diseased fish studied. This bacterium is not thought of as a true marine pathogen but is associated with

diseases of brackish water organisms (turtle, fish, etc.) in eutrophic water (Shotts, et al., 1972).

Brine discharge was found to be either inhibitory or to extend the lag period of growth for the laboratory control microorganisms used in this study. The same discharge, however, promoted the growth of most of the isolates collected in the field. Indeed the amount of growth exhibited by some oil field isolates was proportional to the concentration of brine discharge present in the growth mixture. These results are in agreement with last year's work and it may very well be that the various components of Buccaneer hydrocarbon discharges actually contain nutrients that stimulate growth and biodegradation. Several strains tested showed some ability to attack the alkane hydrocarbons in brine discharge with one strain being particularly effective (B-13).

In contrast to last year's report mixed cultures showed a significant potential for Buccaneer petroleum degradation. This is similar to the work of Zobell and Prokop (1966) who reported 67 to 78% oxidation of Louisiana crude oil by sediment bacteria in an enriched seawater medium for 30 days at 25C. Mixed cultures collected from nature consititute a complex interaction of bacteria. Indeed, the rate of biodegradation by mixed cultures is a function of many factors including the number of types of bacteria in the sample population. Several possible explanations may be suggested as to why Buccaneer field mixed cultures, particularly sediment cultures, seem to be especially effective in degrading the alkane components of BOF crude oil. Many types of bacteria are present in mixed cultures and potentially this variety of bacteria can work together to degrade the complex mixture of hydrocarbons in oil.

Furthermore, mixed cultures are often successful in degrading recalcitrant compounds because one microorganism in the mixture produces a factor necessary for the growth of the degrading organisms.

Utilization of alkanes by different bacterial types from BOF, which had been previously identified by the taxonomic portion of this study, showed no general taxonomic or geographic (oil field area vs. control area) trends. Further studies need to be undertaken in order to provide a sufficient amount of data to indicate the relative rates of microbial degradation for specific strains of BOF isolates. This information could be correlated with the taxonomic profile already done, providing more information on the potential oil-degrading bacteria present in the oil field.

The relative subtle differences between the bacterial oil-degrading capabilities of the oil field and control area may simply reflect the small differences in hydrocarbon concentrations found between the two areas (See Middleditch, et al.,1979). In a high energy area such as the Buccaneer oil field small oil spills are usually rapidly dispersed by wave action and are weathered away by evaporation. Buccaneer field oil-degrading bacteria combined with the abiotic effects should be capable of removing the majority of the oil released normally in Buccaneer brine discharge.

The optimized conditions of the hydrocarbon degradation experiments (e.g. high $NO_3$ and $PO_4$ concentrations) are designed only to estimate the potential degradation ability of the Buccaneer oil field's bacterial population. While an attempt was made to simulate some environmental parameters (e.g. pH and salinity), the enclosure of the sample alone insures higher bacterial numbers than would be found in situ and thus makes data

interpretation difficult. However, the use of uniform experimental conditions should produce comparative measurements that permit at least a rough estimate of microbial degradative capacity.

## CONCLUSION

(1) The bacterial numbers and taxonomic types in the oil field are the same as the control area.

(2) Epiflourescent microscopy is a superior enumeration technique for bacterial populations.

(3) Ninety percent of the bacteria (CFU) in the water column are attached to particles greater than 3 μ in diameter.

(4) More oil degrading and sulfur oxidizing bacteria are present in the oil field than the control area.

(5) Sulfate reducing bacteria are much more common in the sediment at the oil field compared to the control.

(6) The most common taxonomic type in the sediment of the platform and control site were Vibrio, Pseudomonas, Aeromonas, and Acinetobacter.

(7) Bacterial diversity was the same for the control and platform area but changes with the season, being the lowest in the spring.

(8) Aeromonas hydrophila was the only potential bacterial pathogen found associated with diseased fish from the study area.

(9) Presumptive coliform microorganisms were more common near the crew's quarters than the control site but no fecal coliform were found at either site.

(10) Bacterial biomass estimates made from epiflourescent counts were much higher than previous estimates being in the range of $6.2 - 44.0 \times 10^{-6}$ gC/l.

(11) Ninety four percent of the bacteria enumerate show a requirement for salt to grow. This indicates the marine origin of the strains.

(12) Bacterial population from Cruise I & II were different than Cruise III & IV at both the control and platform site.

(13) Brine discharge from the oil field inhibited or retarded growth of laboratory culture but appear to have either no effect or a stimulatory effect on isolates from the oil field.

(14) Both mixed and pure culture from the Buccaneer oil field show the ability to degrade significant portions of the n-alkanes in Buccaneer crude oil.

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